# Role of Medial Hypothalamic Orexin System in Panic, Phobia and Hypertension

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## Abstract

Orexin has been implicated in a number of physiological functions, including arousal, regulation of sleep, energy metabolism, appetitive behaviors, stress, anxiety, fear, panic, and cardiovascular control. In this review, we will highlight research focused on orexin system in the medial hypothalamic regions of perifornical (PeF) and dorsomedial hypothalamus (DMH), and describe the role of this hypothalamic neuropeptide in the behavioral expression of panic and consequent fear and avoidance responses, as well as sympathetic regulation and possible development of chronic hypertension. We will also outline recent data highlighting the clinical potential of single and dual orexin receptor antagonists for neuropsychiatric conditions including panic, phobia, and cardiovascular conditions, such as in hypertension.

## Overview of the Relevant Orexin Circuitry

First described in 1998, the orexins (hypocretins) (ORX/HCRT) is a neuropeptide expressed by a few thousand neurons within the hypothalamus, located across the medial perifornical region (PeF) and dorsomedial hypothalamus (DMH), as well as the lateral hypothalamus (LH) of rodents (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998) and humans (Thannickal et al., 2007). Orexin neurons project extensively to brain nuclei implicated in the control of behavioral state, appetite, and autonomic function (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998). The study of De Lecea reported the isolation of two novel peptides expressed at high levels in the hypothalamus using directional tag PCR subtraction technology. They named these peptides hypocretins based on their hypothalamic localization and weak homology to the secretin/incretin family of peptides (de Lecea et al., 1998). Simultaneously Sakurai and colleagues used a reverse pharmacology approach to identify ligands for the orphan G protein coupled receptor HFGAN72, and named the peptides orexin A (OX-A) and orexin B (OX-B, from the Latin orexis = appetite) since their cell bodies were located within the lateral hypothalamic feeding area and because they stimulated feeding upon intracerebroventricular administration, and the receptor became known as the Orexin 1 receptor (Sakurai et al., 1998). They also described a second receptor for these peptides - Orexin 2 receptor (ORX1 and ORX2; also referred as HCRT-1or HCRT-2). The two isoforms of orexin, A and B, containing 33 and 28 amino acids respectively, are derived from a common precursor, prepro-orexin (de Lecea et al., 1998; Sakurai et al., 1998). Orexin A is non-selective for both ORX1 and ORX2, whereas OX-B is somewhat more selective for ORX2 (Ammoun et al., 2003; Sakurai et al., 1998). ORX1 and ORX2 are strongly conserved across mammals, with 94% identity in the amino acid sequences between humans and rats (Sakurai et al., 1998). ORX1 binds OX-A with high affinity, but it has considerably less affinity for OX-B.

Compatible with the extensive projections of these neurons, ORX has been implicated in a number of physiological functions, including arousal (Harris and Aston-Jones, 2006; Sutcliffe and de Lecea, 2002; Taheri et al., 2002), regulation of sleep (Chemelli et al., 1999; Lin et al., 1999; Sakurai, 2013, 2007), energy metabolism (Burdakov, 2005; Burdakov et al., 2013; Inutsuka et al., 2014), reward-seeking behavior (Harris et al., 2005; Harris and Aston-Jones, 2006; Marchant et al., 2012), behavioral and neuroendocrine responses to stress (Furlong et al.,

2009; Ida et al., 2000), anxiety, panic and fear responses (Bonaventure et al., 2015; Flores et al., 2015; Johnson et al., 2011; Johnson et al., 2012; Pich and Melotto, 2014) and cardiovascular control (Carrive, 2013; Rani et al., 2017). The role of orexin in this diverse range of functions has been reviewed extensively elsewhere (Boss and Roch, 2015; Boutrel et al., 2010; Boutrel and de Lecea, 2008; Huber et al., 2017; Johnson et al., 2012a; Lawrence, 2010; Mahler et al., 2012; Pich and Melotto, 2014; Roberta et al., 2017). In this review, we will highlight research focused on orexin system in the PeF/DMH and the role of this hypothalamic neuropeptide in the behavioral expression of panic, anxiety, fear, and related sympathetic regulation and possible development of chronic hypertension. We will also outline recent data highlighting the clinical potential of single and dual orexin receptor antagonists (SORAs and DORAs) for neuropsychiatric conditions including panic, fear, and cardiovascular disorders.

**Specific panic relevant afferent/efferent connections of orexin**

Two important studies have described the major afferents to the orexin system, one using a genetically encoded retrograde tracer in mice (Sakurai et al., 2005) and the other one a retrograde and anterograde tracers in rats (Yoshida et al., 2006). In 2005 Sakurai and colleagues examined the afferents to the orexin neurons using mice in which the human prepro-orexin promoter drives the expression of a tetanus toxin fragment fused to green fluorescent protein (TTC: GFP). Then, they genetically encoded a retrograde tracer in ORX neurons to determine afferent systems that made synaptic contacts with ORX neurons (Sakurai et al., 2005). This study revealed several brain regions with prominent projections onto ORX neurons relevant in the regulation of panic and fear, including medial prefrontal cortex (mPFC), bed nucleus of the stria terminalis (BNST), nucleus accumbens shell (NAcSh), lateral septum (LS), basal forebrain, basolateral (BLA) and basomedial amygdala (BMA), preoptic area (POA), arcuate nucleus (ARC), midbrain median raphe nucleus (MRN); rostral ventrolateral medulla (RVLM); and neurons in the dorsal motor nucleus of the vagus (DMV).

In addition to confirming many afferent systems identified by Sakurai and colleagues using TTC, in 2006, Yoshida and colleagues identified prominent afferent systems to the ORX system using traditional retrograde and anterograde tracing (Yoshida et al., 2006). Utilizing the cholera toxin B subunit (CTB) they found strong projections from the LS, POA, BNST, and posterior

hypothalamus. They also found robust projections from the NAcSh, BNST, dorsolateral septum, ventral pallidum (VP), central amygdala (CeA), ventral tegmental area (VTA) and dorsal raphe nucleus (DRN), whereas PeF/DMH orexin neurons receive relatively stronger inputs from subiculum, POA, ventromedial (VMH) and anterior hypothalamus (AH), and ARC. These regions are also well known for their role in emotions and autonomic control (Saper, 2004).

On the output side, ORX terminals can be seen not only in the limbic structures described above where they make reciprocal connections, but also in the premotor sympathetic centers of the paraventricular nucleus of the hypothalamus (PVN) (Kannan et al., 2007; Shirasaka et al., 2001; Zhou et al., 2015), as well as in all the autonomic centers of the brain stem, including the periaqueductal gray (PAG), parabrachial nucleus (PBP), nucleus of the solitary tract (NTS) (Ciriello et al., 2013; de Oliveira et al., 2003; Shih and Chuang, 2007; Yang et al., 2003), rostral ventrolateral and ventromedial medulla (RVLM, RVMM) (Huang et al., 2010; Lee et al., 2015; Shahid et al., 2012) and medullary raphe (Nambu et al., 1999; Peyron et al., 1998). Thus, all these neuroanatomical data suggest that ORX neurons are ideally linked with known anxiety and panic brain regions to integrate a variety of stress-associated sensory signals which are also involved in regulating cardiovascular and sympathetic activity. These areas are responsible to mobilize adaptive behavioral and physiological response to restore homeostasis during threat conditions.

**Distribution of orexin receptors in panic relevant regions**

The ORX neuronal projections are present throughout the brain. In many regions of the brain, the expression of ORX1 and ORX2 receptors is co-expressed (Marcus et al., 2001; Trivedi et al., 1998). Yet many other areas have selective expression of the ORX2 or ORX1. The differences in the distribution of ORX1 and ORX2 has been extensively reviewed elsewhere (Johnson et al., 2015, 2012a). **Table 3** below provides a brief summary of their distribution in areas of the brain that mobilize different components of a panic response:

**Table 1: Distribution of the two different ORX receptors (ORX1 and ORX2) in regions critical in the regulation of panic and autonomic responses**

| Region | ORX1 | ORX2 | Key Reference |
|---|---|---|---|
| Medial prefrontal cortex (mPFC) | ++ | ++ | (Gabbott et al., 2005) |
| Lateral Septum | ?- | ++ | (Bakshi et al., 2007; Henry et al., 2006; Sartor and Aston-Jones, 2012) |
| Central amygdala (CeA); Basolateral amygdala (BLA) | ++ | ?+ | (Nambu et al., 1999; Yoshida et al., 2006) |
| Bed nucleus of the stria terminalis (BNST) | ++ | ?+ | (Duvarci et al., 2009; Lee et al., 2008; Sahuque et al., 2006) |
| Paraventricular hypothalamus | ++ | ++ | (Cluderay et al., 2002; Trivedi et al., 1998) |
| Tuberomamillary nucleus | - | ++ | (Marcus et al., 2001) |
| Periaqueductal gray (PAG) | ++ | ++ | (Yoshida et al., 2006) |
| Parabrachial and Kölliker-Fuse nucleus; | + | ++ | (Tokita et al., 2009; Yokota et al., 2016) |
| Dorsal raphe nucleus (DRN) | ++ | ?+ | (Lowry et al., 2005) |
| Locus coeruleus (LC) | ++ | ?- | (Itoi and Sugimoto, 2010) |
| Nucleus tractus solitarious (NTS), medullary autonomic centers (RVLM, RPa, DMNV) | ++ | ++ | (Dampney et al., 2005; McDowall et al., 2006) |

++: express orexin receptors; -: not express orexin receptors; ?+: maybe express orexin receptors;?-: maybe not express orexin receptors.

## Role Orexin in Panic and Phobic Disorders

Preclinical studies indicate that exposure to acute stressors (physiological and psychological) activates ORX neurons and that this is associated with the expression of panic, anxiety, and stress-related behaviors (Furlong et al., 2009; Johnson et al., 2012, 2010; Johnson et al., 2015; Li et al., 2010). The role of orexins (OX-A and OX-B) and orexin receptors (ORX1 and ORX2) in complex emotional behavior including anxiety sensitivity, panic and fear associated learning is emerging (Boss and Roch, 2015; Flores et al., 2015; Johnson et al., 2010). For instance, optogenetically stimulating ORX neurons in rats increases anxiety-like states by activating anxiety-related neural circuits (Heydendael et al., 2014), as well as stress hormone release and

tachycardia (Boss and Roch, 2015). In addition, artificially increasing OX-A levels in the cerebrospinal fluid of rodents increases anxiety associated behaviors (Suzuki et al., 2005), which is consistent with elevated ORX levels being associated with increases in anxiety symptoms in neuropsychiatric patients (Johnson et al., 2010).

Rodent studies have demonstarted that stimulating the PeF and DMH hypothalamic regions with microelectrodes elicited components of the "fight or flight" response such as increases in blood pressure, tachycardia and hyperventilation (Duan et al., 1994; Markgraf et al., 1991) and flight associated locomotor behavior that increased with intensity of the stimulation (Duan et al., 1996). More selective pharmacological studies using discrete hypothalamic microinjections (that do not stimulate fibers of passage), showed that stimulating or disinhibiting the PeF/DMH [with excitatory amino acids or the GABAA receptor antagonist bicuculline methiodide (BMI), respectively] initiate similar panic associated "fight or flight" responses (e.g., pressor responses, tachycardia and increases in locomotion (Anderson and DiMicco, 1990; Samuels et al., 2002; Shekhar et al., 1990; Shekhar and Dimicco, 1987; Soltis and DiMicco, 1992). Site-specific stimulation of adjacent structures such as the LH (Shekhar and Dimicco, 1987) or regions dorsal to the PeF/DMH do not result in any cardiovascular response [see review (DiMicco et al., 2002)]. This pattern of autonomic and respiratory responses is similar to responses observed during panic attacks (PAs) in humans (Liebowitz et al., 1986) and deep brain stimulation of the posterior hypothalamus (that contains the PeF) of humans also leads to tachycardia and self-reported 'panic' (Rasche et al., 2006; Wilent et al., 2010).

Importantly, chronic disinhibition of the PeF region with blockade GABA synthesis utilizing chronic infusions of a glutamic acid decarboxylase inhibitor l-Allyglycine (l-AG, and not its inactive isomer d-AG) produces rats that are vulnerable to displaying panic-like responses to interoceptive stimuli, such as NaLac (Johnson and Shekhar, 2006; Shekhar et al., 1996; Shekhar and Keim, 1997) and hypercapnic gas [see review (Johnson and Shekhar, 2012)], stimuli which also reliably provoke PAs in subjects with panic disorders (PD), a chronic and disabling type of anxiety disorder (Gorman et al., 1994; Pitts and Mcclure, 1967; Woods et al., 1988). This rodent models of panic vulnerability has been extensively documented and has robust face, postdictive, predictive, and construct validity [see review (Johnson and Shekhar, 2012) and (Johnson et al., 2010, 2008; Johnson and Shekhar, 2006; Molosh et al., 2010; Shekhar et al., 1996; Shekhar and

Keim, 1997)]. The PeF panic model, since its inception in 1996 (Shekhar et al., 1996), has consistently proven to be sensitive to interoceptive stimuli, such as NaLac and CO2 [see review (Johnson and Shekhar, 2012)] that provoke PAs in subjects with PD (Gorman et al., 1994; Pitts and Mcclure, 1967; Woods et al., 1988). The sensory pathways which are critical for NaLac response (Molosh et al., 2010; Shekhar and Keim, 1997), anticipatory anxiety behaviors, and cardio-respiratory and sympathoexcitatory circuits have all been well mapped (Johnson et al., 2008). Further studies have demonstrated that the ORX neurons in the PeF play a critical role in generating panic responses in this model (Johnson et al., 2010). Orexin neurons increase their firing rate in response to changes in CO2 and lactate concentration in the brain stem. Using CO2 gas challenge or administration of the anxiety provoking agent sodium lactate, and ORX neurons in the PeF/DMH region appear to be critical to eliciting coordinated behavioral and physiological panic-like responses (Johnson et al., 2012, 2015, 2010). Further evidence of some orexin role in panic is supported by that the Val308Iso allele of the ORX2 gene has been found to be associated with panic disorder (Annerbrink et al., 2011).

While PAs are necessary for diagnosis for PD, approximately 50% of PAs are expected and occur in situations where an external cue is associated with a past experience of PA or stressful environment (Shulman et al., 1994). Agoraphobia, where people with PD begin to avoid situations that are associated with PA, is estimated to affect up to 50% of those with PD (Kessler et al., 2006), resulting in restricted lifestyle and impairment in occupational and interpersonal functioning (Kessler et al., 2006; Noyes, 2001). The mechanism leading to agoraphobia is thought to be similar to conditioned fear, an amygdala dependent learning process, where the unconditioned aversive experience (US, i.e., the PA) gets conditioned to elicit fear in the associated neutral context or stimulus (CS, i.e., places, activities, environmental contexts, etc.) (Lissek et al., 2010). In conditioned fear paradigms comparing PD patients to controls, no differences were observed with the acquisition of fear conditioning, but PD patients displayed reduced ability to extinguish the conditioned fear response (Michael et al., 2007) and overgeneralized fear responses to stimuli similar to the CS compared to controls (Lissek, 2012; Lissek et al., 2010). The molecular mechanism underlying this extinction deficit, which appears to be critical for developing agoraphobia in PD patients, is still largely unknown. In this context,

ORX was recently shown to enhance amygdala based fear conditioned behaviors (Flores et al., 2014; Sears et al., 2013) via their projections to both the BLA and CeA (Peyron et al., 1998).

In a recent study (Molosh, 2018), we demonstrated that panic-prone rats with chronic disinhibition of PeF neurons demonstrate delayed extinction of conditioned fear. This is similar to PD patients who are reported to have normal acquisition of conditioned fear, but show greater resistance to extinguishing those conditioned fear responses (Michael et al., 2007). In the same study, we also determined that panic-prone state is associated with altered network properties of reduced inhibition and enhanced excitation in the BLA, along with disruptions of select GABA and glutamate genes expressions in the BLA and CeA (Molosh et al., 2018; also see **Figure 1**). Particularly, expression of metabotropic glutamate type 2 receptor (mGluR2) gene was significantly reduced in the CeA of panic-prone rats. Treating such panic-prone rats with a selective mGluR2 positive allosteric modulator (PAM) blocked the panic-responses following 0.5M NaLac and normalized fear extinction deficits (Molosh et al., 2018). Such pre-clinical evidence suggests that chronic activation of ORX neurons in the PeF could lead to altered fear network in the amygdala as a key factor in developing persistent fear responses and development of phobia. This concept was further confirmed by supportive human clinical data from a post-hoc analysis of a proof-of-concept depression clinical trial investigating the efficacy of the mGluR2 PAM compound where in the subset of participants with comorbid panic disorder, treatment with this mGluR2 PAM resulted in complete remission of panic symptoms (Molosh et al., 2018).

## Role Orexin in Hypertension

### Orexin in the regulation of stress-induced hypertensive response

Various studies have reported that central administration of orexins increases the mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (SNA), and plasma catecholamine levels in conscious rats, indicating that orexin activates the sympathetic nerve activity and cardiovascular function to regulate the blood pressure (Samson et al., 1999; Shirasaka et al., 1999; Lin et al., 2002). At the circuit level, injection of OX-A and OX-B into the NTS has been shown to modulate systemic arterial pressure and HR (Shih and Chuang, 2007). Huang and colleagues also reported equal ability of OX-A and OX-B in depolarizing RVLM

neurons in brainstem slices (Huang et al., 2010). Pressor responses elicited from disinhibition of the PeF/DMH can be attenuated by microinjecting the $GABA_A$ receptor agonist muscimol into the RVLM (Fontes et al., 2001). The number of immunoreactive OX-A neurons in the PeF/DMH area as well as the protein expression of ORX1 in the RVLM was greater in stress induced hypertension rats compared to control rats (Xiao et al., 2013). Another study suggests that enhanced ORX2-nNOS signaling in the RVLM contributes to hypertension in spontaneously hypertensive rats suggesting nNOS may be an important signal pathway for the ORX system in the RVLM in several forms of hypertension (Lee et al., 2015).

Li and colleagues demonstrated that both ORX1 and ORX2 participate in the pathogenesis of high blood pressure in spontaneously hypertensive rats (Li et al., 2013). In this study, the blockade of ORX receptors using potent dual ORX receptor antagonist, almorexant, markedly lowered the blood pressure, HR, and noradrenaline levels in cerebrospinal fluid (CSF) and plasma of adult spontaneously hypertensive rats as compared to normal rats, suggesting the role of both ORX1 and ORX2 in the pressure response (Li et al., 2013). However, considering that Li and colleagues used a dual receptor antagonist, the results may not be sufficient to clarify if the reduction of blood pressure resulted from the blockade of one receptor or both. On the other hand, another study demonstrated that ORX2 involvement may be critical in the pathophysiology of hypertension in anesthetized SHR (Lee et al., 2013). In contrast, Shih and coworkers reported the cardioexcitatory, as well as cardio-depressive effect of orexins (Shih and Chuang, 2007). This study reported that bilateral microinjection of OX-A or OX-B into the NTS evoked bidirectional cardiovascular effects in a dose-dependent manner. Administration of an ORX1 antagonist (SB-334867) or ORX2 antiserum completely abolished the bidirectional cardiovascular effects, suggesting the dual role of orexins in regulating pressure response (Shih and Chuang, 2007).

Using the immunohistochemical technique, another study also revealed that BPH/2J mice (a genetic model of hypertension) have 29% more ORX neurons as compared to BPN/3J (normal) mice in the lateral hypothalamus (Jackson et al., 2016). The hypertensive role of the orexinergic signaling in BPH/2J mice was confirmed as the systemic injection of an ORX1/2 antagonist; almorexant significantly attenuated the blood pressure in BPH/2J mice, suggesting that the enhanced central orexinergic system is a major contributing factor to hypertension in this model

(Jackson et al., 2016). Moreover, Beige and coworkers have reported that the cardiovascular depressive effect of the combination of selective ORX antagonists (ORX1 and ORX2) was greater and closer to that of the almorexant (dual orexin receptor blocker) in anesthetized rats, suggesting the contribution of both the receptors in evoking cardiovascular response from the PeF (Beig et al., 2015b). These findings support the notion that the ORX system is involved in cardiovascular responses to certain forms of stresses (Beig et al., 2015a; Furlong et al., 2009; Johnson et al., 2010) and genetic models of hypertension (Jackson et al., 2016; Lee et al., 2013; Li et al., 2013).

**Orexin and obesity-related hypertension**

The ORX system may also be a link between obesity and hypertension. Central administration of ORX stimulates feeding in rats (Sakurai et al., 1998). Furthermore, ORX neurons are sensitive to peripheral metabolic signals specifically they are inhibited by leptin and excited by ghrelin (Yamanaka et al., 2003). Alterations in the ORX system have also been implicated in the obese Zucker rat model of obesity-related hypertension (Zhou et al., 2015). Augmented sympathetic tone is thought to contribute to hypertension in this model (Carlson et al., 2000).

To investigate the contribution of the ORX system to obesity-induced hypertension, Zhou et al. first compared the protein levels of ORX1 and ORX2 in the PVN of Zucker rats and found that PVN ORX1 expression was significantly greater in 15-week-old obese rats compared to lean rats (Zhou et al., 2015). Administration of the ORX1 antagonist SB-334867 was associated with concomitant reductions in the activation of PVN neurons (Heydendael et al., 2011; Vanderhaven et al., 2015) and reduced ACTH release (Heydendael et al., 2011). Consistent with this result, microinjection of the ORX1 antagonist SB334867 into the PVN significantly decreased MAP and renal SNA in obese Zucker rats but not in lean Zucker rats (Zhou et al., 2015). PVN blockade of ORX2 did not affect MAP or renal SNA in obese Zucker rats or lean Zucker rats. Additionally, OX-A induced a greater increase in the firing rate of spinal cord projecting PVN neurons in obese Zucker rats compared to lean Zucker rats, which was attenuated by ORX1 antagonist SB334867, but not ORX2 antagonist TCS OX2 29 (Zhou et al., 2015). These studies suggest that upregulation of the ORX1 contributes to increased firing of PVN neurons and

contributes to augmented sympathetic nerve activity and hypertension in the obese Zucker rat model.

Our recent studies also suggest that adolescence high fat diet (HFD; consumption of 45% HFD for 9 weeks,) leads to not only obesity, hypertension, autonomic reactivity, and metabolic syndrome, but also panic-like responses to 7.5% CO2. Animals subjected to chronic HFD also demonstrated hyperactive ORX neurons (as measured by c-Fos) in DMH/PeF, a mechanism that is likely contributing to the panic vulnerability, sympathetic reactivity, and hypertension (Abreu et al., unpublished data).

Finally, the role of orexin in regulating sleep wake cycle is another key mechanism by which it can further modulate stress responses. Sleep deprivation and sleep disorders are associated with maladaptive changes in the HPA axis, leading to neuroendocrine dysregulation. Excess of glucocorticoids increase glucose and insulin and decrease adiponectin levels. Many studies have shown an association between sleep duration and obesity both in adults and children, suggesting that short sleep duration may be a predictor of weight gain (Patel and Hu, 2008) and an important risk factor for development of insulin resistance, diabetes, and cardiovascular disease (Spiegel et al., 2004, 1999; Taheri, 2006). These observed changes due to sleep loss indicate a probable imbalance between food intake and energy expenditure caused by neuroendocrine alterations.

## Opportunity for Treatments Based on Orexin Antagonists

As noted above, ORX1 and ORX2 are differentially distributed throughout the mammalian brain (Marcus et al., 2001). As a rough generalization, the ORX1 to ORX2 expression ratio is higher in the panic, fear, and stress-induced hypertension circuitry, such as the PeF/DMH, PVN, BNST, amygdala, and LC (Marcus et al., 2001; Trivedi et al., 1998). The differences in ORX receptor distribution, together with differences in functional profiles of these networks, support a rationale for selectively targeting either ORX1 or ORX2 for different clinical indications.

This increased understanding of the biology of the ORX system has promoted several drug discovery programs, and such efforts are leading to the development of compounds with different selectivity (Faedo et al., 2012; Lebold et al., 2013). While the role of the ORX2 receptors in sleep and arousal is strongly supported by the available experimental

evidence (Gatfield et al., 2010), the therapeutic potential and disease targets of selective antagonism of the ORX1 receptors is still developing (Gotter et al., 2012). In rodents, the autonomic and behavioral responses to stress are attenuated by pre-treatments with ORX1 antagonists, such as SB334867 (Johnson et al., 2012b, 2010), the first pharmacological tool used as ORX1 antagonist (Jones et al., 2001; Smart et al., 2001); GSK 1034865 (Gozzi et al., 2011); 2,5di-substituted piperidines (Jiang et al., 2012); and ACT-335827 (Steiner et al., 2013). Some compounds have successfully progressed into human trials, in particular, the dual ORX1-ORX2 antagonists (DORAs). Almorexant (ACT-078573) (Hoever et al., 2012; Steiner et al., 2012), SB649868 (Bettica et al., 2012), and suvorexant (Herring et al., 2012) have all been administered to human subjects. However, so far only suvorexant (MK-4305) has been approved by the US Food and Drug Administration (FDA) in 2014 as a drug to treat insomnia (Coleman et al., 2017).

The selective ORX1 antagonists are still under development. Many ORX1 antagonists are effective at reducing anxiety-like behaviors following different stressors (Plaza-Zabala et al., 2010; Staples and Cornish, 2014; Vanderhaven et al., 2015). Our group has shown that ORX1 antagonists also attenuate panic-like responses following panicogenic challenges like CO2, sodium lactate or anxiogenic drugs (Johnson et al., 2012), (Johnson et al., 2010). Recently, using a CO2-panic provocation model, we have tested a dual ORX1/2 antagonist (DORA-12) along with a highly selective ORX1 antagonist (Compound 56) or ORX2 antagonist (JNJ10397049) to assess ORX1 and ORX2 efficacy in the treatment of panic and anxiety (Johnson et al., 2015), alongside alprazolam, a benzodiazepine positive control. Both DORA-12 and Compound 56 were effective in treating the panic and hypertensive responses similar to alprazolam, whereas JNJ10397049 was not effective. Such results strongly support the development of selective ORX1 antagonists (SORA1s) for treating panic and stress induced cardiovascular symptoms (Johnson et al., 2011; Johnson et al., 2012).

## Conclusion

Orexin is critical for promoting adaptive physiology and behaviors in response to stressful and anxiety-provoking stimuli. This is accomplished by interactions between the ORX neurons and key stress-sensitive, as well as arousal and autonomic regulation regions of the brain. However, these adaptive responses can, in the face of susceptible biology, and chronic or repeated stressful stimuli, become dysregulated, manifesting as maladaptive behaviors and disorders. While much

further research is still required, most evidence to date points to a relationship between hyperactive ORX neurons in stress, chronic sympathetic arousal, and panic phenotypes. Thus, ORX receptor antagonists may represent a potential therapeutic option for the treatment of panic-related disorders as well as some forms of hypertension.

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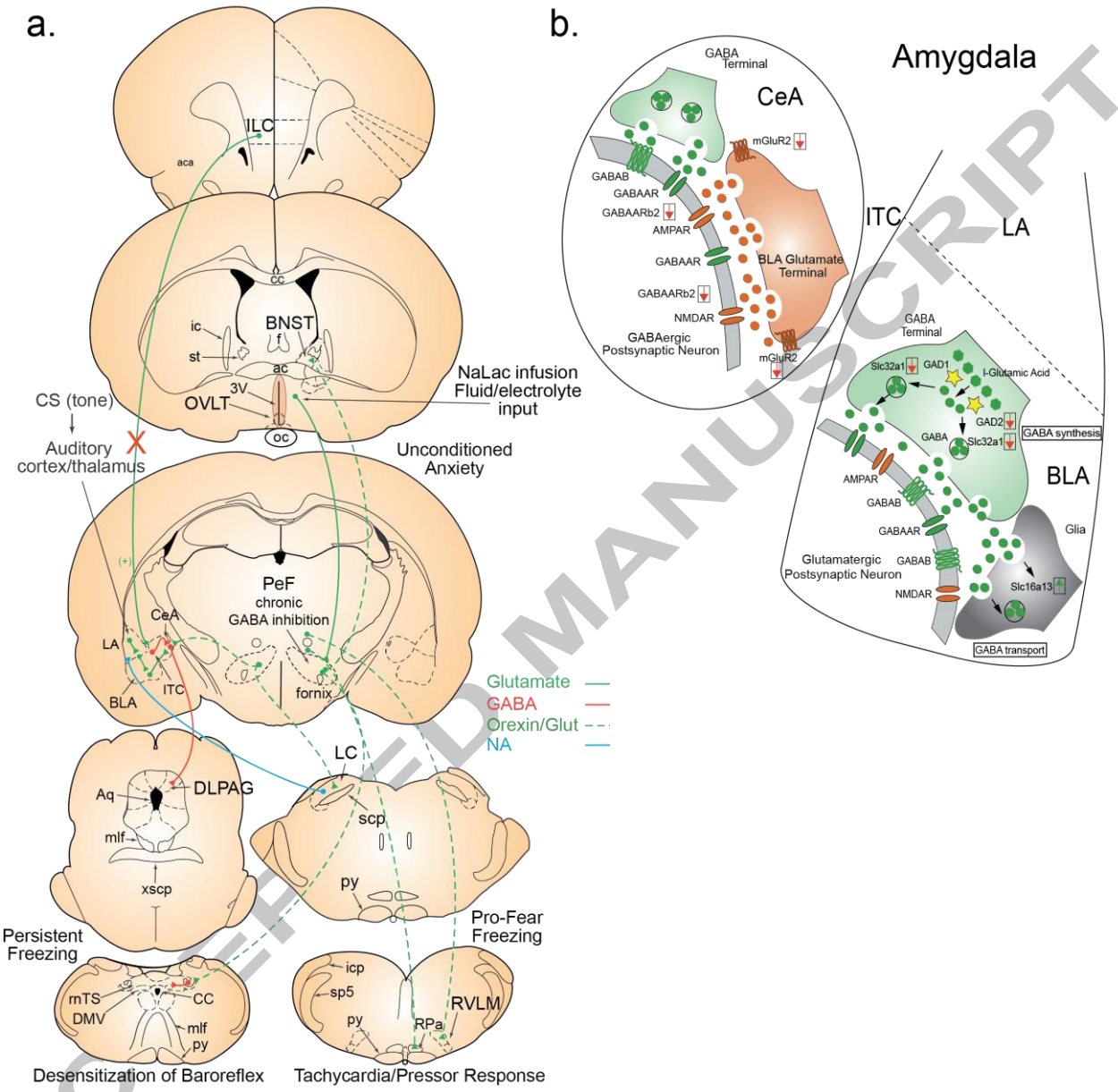


**Figure 1.** The proposed (a) CNS network for ORX regulation of panic and sympathetically mediated hypertensive responses; and (b) amygdala hypothetical neurotransmitter mechanisms that contribute to panic and phobia symptoms in PeF ORX activation model of panic (Molosh, 2018). **1b**. Chronic disinhibition in PeF/DMH leads to shift of excitatory/inhibitory balance towards excitation in the amygdala region. In the BLA, the reduction of GAD2 gene likely causes overall reduction of production of GABA from l-glutamic acid (GABA synthesis) (Chao et al., 2010). Additionally, we observed a significant reduction of Slc32a1 gene, that encodes GABA vesicular transporter, which involved in

GABA and glycine uptake into synaptic vesicles and thus could result in attenuated synaptic release of GABA (Wojcik et al., 2006; Yamada et al., 2012). Finally, increased Slc6a13 gene expression, which encodes sodium-dependent GABA and taurine transporter, may also enhances synaptic GABA re-uptake from synaptic terminals (Borden et al., 1995)(Conti et al., 1999). Interestingly, Slc6a13 under normal conditions has low expression and effect on GABA clearance in the CNS (Zhou and Danbolt, 2013), however, under pathological conditions Slc6a13 has been demonstrated to have increased CNS expression that contributes to GABA clearance (Paul et al., 2014). In the CeA, we observed a significant reduction of the benzodiazepine-sensitive GABAA receptor subunits beta-2 (GABAARb2), which most likely causes alterations of tonic inhibitory current (Herd et al., 2008). Moreover, decrease of mGluR2 receptor gene may reduce the presynaptic control of glutamate release and increase of excitatory tone in the CeA (Bradley et al., 2000; Lovinger and McCool, 1995; Rainnie et al., 1992).

Abreu et al.
**Role of Medial Hypothalamic Orexin System in Panic, Phobia and Hypertension**

Highlight for Review

In this review, we will highlight research focused on orexin system in the medial hypothalamic regions of perifornical (PeF) and dorsomedial hypothalamus (DMH), and describe the role of this hypothalamic neuropeptide in the behavioral expression of panic and consequent fear and avoidance responses, as well as sympathetic regulation and possible development of chronic hypertension. We will also outline recent data highlighting the clinical potential of single and dual orexin receptor antagonists for neuropsychiatric conditions including panic, phobia, and cardiovascular conditions, such as in hypertension.